# FABRICATION OF A CELL-BASED BIOSENSOR USING GREEN TAPE CERAMICS WTIH HUMAN EMBRYONIC KIDNEY AND SEA URCHIN EGG CELLS

Salme DeAnna Burns - Mechanical Engineering at the University of Pennsylvania
Engineering Advisor: Dr. Haim Bau (University of Pennsylvania)
Biology Advisor: Dr. Jacqueline Tanaka (Temple University)
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## ABSTRACT

Cells are electrically active, thus fabrication of a cell-based biosensor will contribute much information about how cells respond to different environments. With such a device, researchers can submit various types of cells to different forms of stimulus and observe their reaction. A biosensor was designed and fabricated out of DuPont Green Tape ceramics and thick film conductor materials, working with human embryonic kidney cells in order to test the compatibility of cells with various types of materials. t was found that DuPont's gold paste conductor is harmful to cells and that pure gold is needed in order for cells to attach and live on the sensor's electrodes. Next, sea urchin eggs were used. These eggs are larger and more robust than most cells. A device containing channels with two pure gold electrodes covered with a thin insulating layer of Teflon was constructed. A small portion of the Teflon removed over each electrode provides the necessary small area needed for an electrode that is used to measure the electrical activity of cells. Although I was unable to gain many meaningful electrical measurements, an improved sensor that is constructed out of more compatible materials should be constructed and used to test a cell's electrical response to a change in environment.

## 1. INTRODUCTION

### 1.1 LONG-TERM GOALS

A cell-based biosensor has many intriguing implications in the biomedical field. Most notably, it can help us to understand how various environments can affect our cells. A cell-based biosensor will have the capability to observe changes in the electrical activity of cells. A cell can act as a sensor because cells are highly sensitive to their environment. Thus, as a cell is subjected to a specific surrounding, the corresponding change in the cell's electrical activity can be measured. Clearly, an appropriate electrical device needed to measure these electrical changes can provide a vast amount of information. In my research in particular, I wanted to test specific materials needed to construct the device with cells as well as to design and fabricate a device that would be suitable for making the electrical measurements. Once this device is perfected in the

future, doctors and researchers will be able to gain a greater understanding of many different types of cells in various environments, both helpful and harmful.

## 1.2 ELECTRICAL ACTIVITY OF CELLS

Cells have the ability to be excellent sensors because of their electrical activity. This electrical activity arises from an electrical potential across a cell membrane. A cell membrane is made up of a semi-permeable phospholipid bilayer. It is semi-permeable because the membrane is only permeable to small, non-polar or uncharged molecules. However, it is impermeable to larger molecules and ions. Nevertheless, these molecules and ions can be selectively transported across the membrane via proteins that act as channels or carriers. See Figure 1 for a schematic of a cellular membrane.

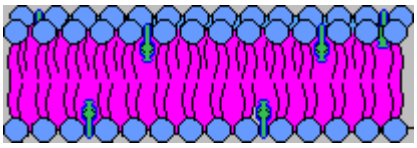

Figure 1: Phospholipid bilayer

The ions that cause the electric potential across the membrane are primarily sodium ($Na^+$), potassium ($K^+$), and chloride ($Cl^-$). Because both chemical and electrical forces must be simultaneously balanced, a negative potential on the inside of the cell with respect to the surroundings is established. This potential difference is called the resting potential of a cell [1].

Although a cell has a resting potential, there are times when the potential difference across a cell changes in a direct response to a change in the permeability of the membrane. This change in permeability occurs due to a stimulus, which may come in the form of a foreign chemical in the cell environment, shear stresses, electrical pulse, etc. When the stimulus reaches a certain level, the permeability suddenly changes and an action potential occurs (see Figure 2).

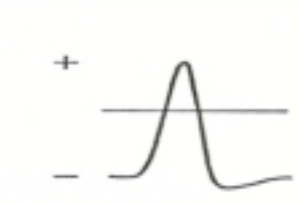


Figure 2: Action potential

As seen, there is a depolarization such that the inside of the cell becomes positive with respect to the environment, and then a repolarization occurs. Note that the size of an action potential does not correlate with the intensity of the stimulus. Rather, once a

threshold stimulus is met an action potential occurs. It does not increase in size as the stimulus is further increased [1].

Another way to observe the electrical activity of a cell is through impedance measurements, which is measured when applying an alternating current. A cell membrane essentially acts as a resistor and capacitor connected in parallel (Figure 3).

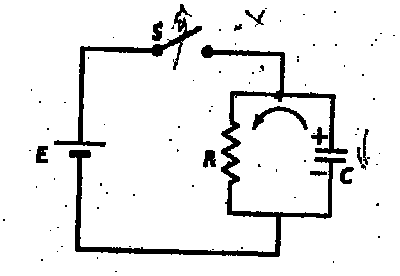


Figure 3: Electrical analog of the cell membrane

Impedance is a quantity relating voltage to current, and is dependent on both the capacitative and resistive qualities of the membrane. It is also dependent on the frequency of the applied current [1].

There are several ways to employ cell impedance when using the cell as a sensor. For instance, if a stimulus is applied, the cell is affected if the impedance measurement changes. This change in impedance occurs due to cell motility. When cells are sufficiently stimulated they may move away from the testing electrode or even die. Thus, as the electrode comes in contact with lower or higher density of cells depending on the stimulus, the impedance will change[2].

## 1.3 BACKGROUND IN DUPONT GREEN TAPES

DuPont Low Temperature Cofire Ceramic System Material (here after referred to as Green Tape or ceramic) was used to fabricate the biosensor. Green Tape is pliable and easy to work with prior to firing; it can be simply slit or cut into the desired shape. Several layers can be utilized and laminated together in order to produce the appropriate product. Within the layers, DuPont thick film conductors are used for the interconnecting pathways (lines and vias). Once the product is finished, the Green Tape is cofired with the thick film conducting pastes. During firing the product has approximately 12% X-Y shrinkage. Both the pastes and the Green Tape have matched shrinkage. Post-firing, the product is rigid.

### 1.3.1 LAMINATION

Lamination is the process of securing two or more layers of Green Tape via a hydraulic press. The layers are lined up appropriately and placed in the press. The surfaces of the press are at approximately 70°C and the applied pressure should equal 3,000 psi. The layers should remain at these conditions for five minutes and then be rotated 180 degrees for another five minutes. Lamination can be done with the desired

amount of layers in order to increase thickness or strength. It is also done to secure layers that have been previously processed with the thick film pastes. Before firing, all layers should be secured together.

### 1.3.2 COFIRING

Processed and laminated Green Tape is placed into a programmable oven such as the Fisher Scientific Isotemp Muffle Furnace [3]. As Figure 4 shows, the temperature is raised from room temperature to 350°C at 10° per minute, held at 350°C for 45 minutes, stepped to 850°C at 10° per minute, held at this temperature for 30 minutes, and then finally the temperature returns to room temperature.

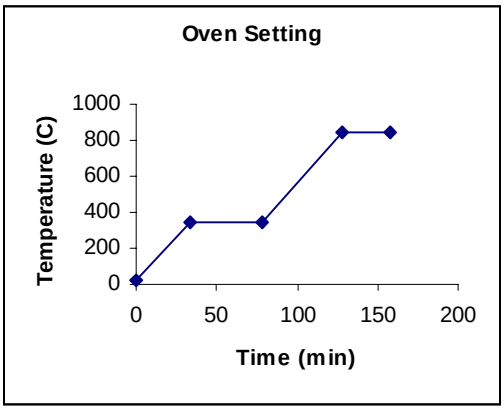


Figure 4: Cofiring schematic (final cooling is left off since it take several hours)

For more information see the Green Tape Material System Design and Layout Guidelines for Prototypes [4] or contact Dupont.

## 2. METHODS

This research has combined engineering and biology so as to simultaneously fabricate a cell-based biosensor. As mentioned above, the biosensor is fabricated out of Green Tape ceramics, which is cheaper than its counterparts, e.g. silicon, and it is easy to work with since the material is pliable in the unfired state. Several layers can be laminated together, resulting in an easily fabricated conducting pathway from the electrodes to the interfacial are. Gold was initially chosen for the electrodes since cells presumably will attach to gold surfaces [2], while Teflon was chosen to cover the channels. Teflon is an insulator and allows for easy handling of the gold paste. In other words, an electrode could be laid in any shape or size. Then a small portion of the Teflon could be removed to expose the appropriate amount of electrode area needed.

Human kidney embryo cells were initially chosen as the cell sensor because they were readily available. These cells are very sensitive to their environment and would hopefully be easily stimulated. They also grow in close contact to one another while spreading on the surface of contact. Testing the various materials needed to construct the

device by observing if the cells are dead or alive would reveal if the cells are compatible with the material. In other words, if the cells are floating, then the material has killed them and should not be used. If the cells are attached to the bottom of the petri dish then the material can be observed under a microscope to tell if the cells are attaching to the material.

Sea urchin eggs were later used as the sensor. These cells were chosen as the next step for several reasons. First, they are much larger than the kidney cells. The diameter of a sea urchin egg is approximately 100 μm while a kidney cell is closer to 5 μm. The larger size would aid in actually visualizing the egg and how it attached and responded to the materials in the device. Additionally, the eggs are strong in comparison to other cells. Since they must naturally survive until fertilization, they would be able to survive after removal from the female urchin until experimentation.

## 2.1 FABRICATION OF MATERIAL SAMPLES

First, small sample chips were fabricated in order to explore how cells would react and behave with the materials. Two layers of the ceramic material were laminated together. After lamination, the material was cut into small chips approximately 12 x 12 mm to get a final chip size of 10 x 10 mm after shrinkage. Of the cut pieces, two-thirds were left uncoated and the remaining one-third were coated in gold paste (DuPont product number 5734). These pieces were then simultaneously cofired.

Because Teflon coating of the chips is not a cofiring process, Teflon was applied to half of the bare chips post-firing. After application of the Teflon, the ceramic and Teflon must be fired at 350° C for 30 minutes in order for the Teflon to dry.

After many applications of cells to these chips, new chips were fabricated using a pure gold coating as opposed to the gold paste. This was done via evaporating a very thin layer of gold onto a post-fired ceramic chip. This layer was about 0.1 μm thick. Additionally, pure gold was deposited on glass cover slips.

## 2.2 CARING FOR HUMAN EMBRYONIC KIDNEY CELLS

Human embryonic kidney cells are small cells about 5 μm in diameter, that prefer to adhere to a surface in order to grow and multiply. These cells grow in close contact with one another and when alive look flat. The cells in Figure 5 are not completely healthy cells. When the cells become rounded instead of flat and attached, it is an indication that they are unhealthy and dying.

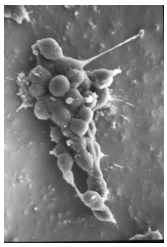

Figure 5: Human embryonic kidney cells on gold surface
visualized on scanning electron microscope at 1250x magnification

In caring for the cells one must first raise a batch of frozen cells. The cell medium (DMEM with 10% FBS) is warmed to 37°C in a warm bath. When the bath reaches this temperature, the cell vial is put into the bath in order to allow for the cells to rapidly thaw. Once unfrozen, the cells are placed in a 15 ml test tube with 8 ml of medium. Subsequently, the cell suspension is centrifuged for three minutes. The supernatant is discarded and the cells are re-suspended in 1 ml of medium. Finally, the cells are added to a flask containing 5 ml of medium. The cells are kept in an incubator at 37°C with 5% carbon dioxide.

In order to keep the cells alive they need to be split every few days. Although, the human embryonic kidney cells need to grow in close proximity to one another, they also need to adhere to the bottom. Thus, when the flask becomes overpopulated, the cells begin to die. Therefore, it is crucial to split cells when they become about 80% confluent. As with raising cells, the medium, along with trypsin, must be warmed to 37°C. For my purposes, I usually split cells into a new flask containing 500,000 cells with 5 ml if medium and small petri dishes containing 300,000 cells with 2 ml of medium each. Initially, I added chips and or samples that I wanted to observe into the bottom of the petri dishes along with the appropriate amount of medium. Once these dishes were prepared, the flask in which the cells were contained was removed from the incubator and the medium and dead cells were discarded. The cells were then rinsed with 3 ml of cold phosphate-buffered saline (PBS) and then the PBS was discarded. Trypsin was added to the flask and the cells were incubated for 3 minutes. The trypsin was used to detach the cells from the bottom of the dish. When the cells were removed from the incubator, the flask was shaken to further remove all attached cells. This suspension was added to 5 ml of medium in a test tube and centrifuged for 4 minutes. Finally the supernatant was discarded and the pellet was re-suspended in 1 ml of medium. The cells were then ready to be counted using a hemocytometer. Finally, the appropriate volume of cell suspension was added to the dishes and flask and kept in the incubator. Cells could only be split 5 or 6 times until they needed to be completely discarded.

## 2.3 SCANNING ELECTRON MICROSCOPE

The scanning electron microscope (SEM) was used in order to visualize the kidney cells on the gold surface. The cells were fixed to the sample surface before viewing by removing the medium and placing a thin layer of glutaraldehyde (2%) covering the cells for 15 minutes. The cells were then washed with Coleman wash to

remove the fixative. Finally, the cells sat for 20 minutes in a series of acetones in order to dry out the cells via the following protocol: 30% acetone, 60% acetone, 90% acetone, and 100% acetone. The 100% acetone washing occurred three times. After the final use of acetone, the cells and samples were allowed to air dry for several minutes and then adhered to a platform that is compatible with the SEM. The samples were sputter coated in gold and then placed into the SEM for observance (see Figure 5).

## 2.4 REMOVAL AND CARING FOR SEA URCHIN EGGS

Sea urchins are spiny sea creatures, as seen in Figure 7. I worked with the Arbacia Punctulata (Purple Urchin), which inhabits the waters off the eastern coast of the U.S. For my purposes, I needed a non-lethal way to provoke the urchin to extrude its eggs. This was done using electrical shock. The sea urchin is placed, mouth down, into a dish with filtered seawater so that the mouth will remain wet. The five gonopores (see Figure 6) are facing up.

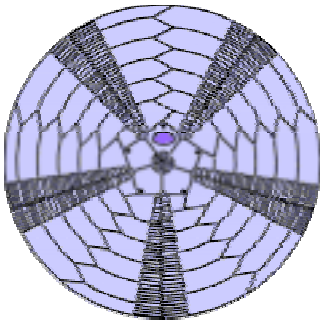

Figure 6: Bottom view

Figure 7: Setup of removal of eggs (left)
Eggs on surface of urchin after shedding (right)

A regular electric cord is striped and split. In order to increase the surface area of the wires, solder is added to the tips of each. The wires are touched to the surface of the urchin on opposite sides. Approximately 25 volts are then applied to the urchin, as seen in Figure 7. If the urchin is female and producing eggs, eggs should be shed through the gonopores within a minute of shocking. This extrusion is dark red and looks like blood. The eggs can be removed from the urchin with a pipette and placed into a container containing filtered seawater. The urchin can then be placed back in her tank and may be used again if needed. The eggs can be stored in a refrigerator for several days until needed. If access to a cold room of 12°C is possible, this environment will keep the eggs

alive for longer. For more information on sea urchins see the following website [5]: http://www.stanford.edu/group/Urchin/contents.html.

The eggs are naturally coated in a protective jelly. With this coat on, the cells look red and round and float without interaction with one another. However, the presence of this jelly coat is not ideal for electrical testing. First, with the jelly coat the cells will not attach to the electrode. Only with this coating removed will the typical phospholipid bilayer of a cell be exposed and hopefully the eggs should then be able and willing to attach to the gold electrodes. Second, it has been shown that this jelly coat provides inaccurate electrical measurements due to the presence of a K+ current [6]. This K+ current occurs because the follicle cells of the jelly layer are electrically coupled to the cell membrane via gap junctions.

To remove this jelly layer, the eggs can be introduced to acidified seawater via the following procedure [7]. A 1 ml egg suspension containing the desired concentration of eggs is microcentrifuged, then the supernatant is discarded and the pellet resuspended in acidified seawater. The seawater should be acidified with acidic acid to pH 4.5. Finally, the microcentrifuge is manually swirled for 5 minutes in order to get a pellet of eggs and a supernatant of jelly coat material. Discard the supernatant and re-suspend the jelly-free eggs in filtered seawater.

In using this protocol, the desired results were not produced exactly. The amount of time the cells were left in the acid and how they were centrifuged (either manually or in the microcentrifuge) after acidification was varied numerous times. No best way to remove the coat was determined. The results were fairly similar after many different techniques were tried. A significant amount of debris and only a few eggs remained each time after treatment in the acidified seawater. The best method that I could carry out was to microcentrifuge the eggs for one minute in the seawater, discard the supernatant and re-suspend the eggs in the acidified seawater, and finally microcentrifuge these eggs immediately for one minute. After re-suspending the eggs in seawater, a significant amount of debris still remained; however, many more intact eggs were also present in comparison to other methods.

## 2.5 FABRICATION OF BIOSENSOR

Figure 8 is a schematic of the step-by-step fabrication of the biosensor. The numbers on the picture correspond to the fabrication steps presented below.

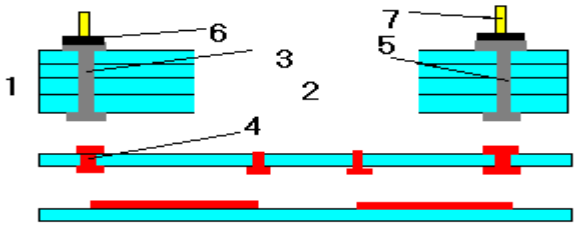


Figure 8: Fabrication steps of the biosensor

1. Four layers are laminated together.
2. The CNC machine is used to cut several channels through the four layers of ceramic. The channels are approximately 0.7 inches by 0.2 inches.
3. Inserting a hole with a needle through the Green Tape material makes the vias.
4. DuPont gold paste, thick film material number 5734, bottom two layers, is applied to the vias manually by "painting" it onto the surface. By applying the material on both sides of the via, the via is automatically filled. The gold paste is also applied to the bottom surface in order to connect the electrodes to the solder pads on the surface of the device.
5. DuPont conducting material number 6141 (through the top four layers) is used to fill the longer vias as in step 4.
6. DuPont silver paste is used as the solder pad material on the surface of the device (number 6146).
7. After all of the layers are laminated together and cofired, gold BNC pins are soldered on the surface in order to make a connection to an amplifier.

Two separate devices were fabricated. One device had electrodes made out of gold paste (see Figure 9), which were applied during step four. The second device had pure gold deposited into the channels after the firing process. After these steps, the channels were covered by Teflon. The Teflon was then fired at 350°C for 30 minutes. Finally, small holes of 125 µm were inserted through the Teflon in order to expose the gold surface beneath. This method provides for a way to have a very small electrode.

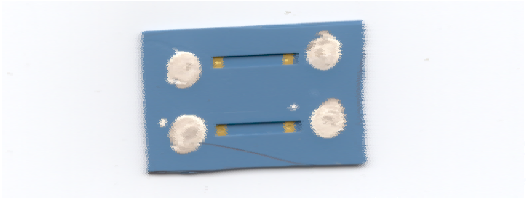

Figure 9: Sensor with two chambers and gold paste electrodes
Gold BNC pins were later connected at the four soldering pads

## 2.6 USING THE DAGAN 8900 PATCH CLAMP AMPLIFIER

The Dagan 8900 Patch Clamp and Whole Cell Clamp was used in order to make preliminary electric measurements. A gold BNC pin on one side of the chamber is attached to the backside of the probe. An external electrode is placed into the chamber. This external electrode is a bare silver wire in an electrode holder. The electrode holder is connected to the 8910 probe as seen in Figure 10. This probe is also grounded to the bath and attached to the gold BNC pin on one side of the chamber by the screws on the backside of the probe. Within the probe case there is a high-speed low noise current to voltage converter.

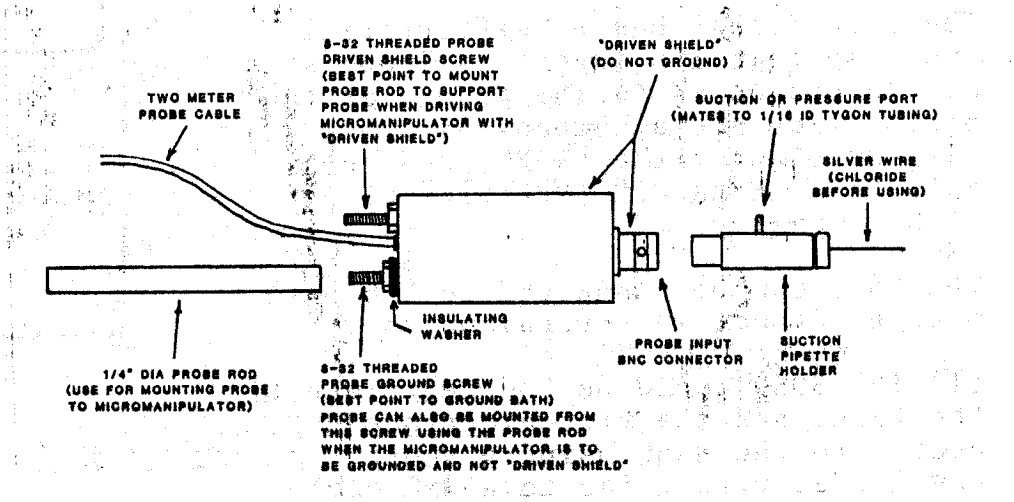


Figure 10: 8910 Probe

A voltage or current clamp can be set up. I used a voltage clamp. See the manual for details in using the current clamp. The switch on the right of Figure 11 controls whether the voltage or current is clamped. Additionally, a test pulse can be activated (see left of figure). While in the voltage clamp mode, the test pulse selects the amplitude of the square wave voltage. If the test pulse is set too high, then the amplitude of the voltage is 20 mV, while if it is set too low, the amplitude is 20 μV.

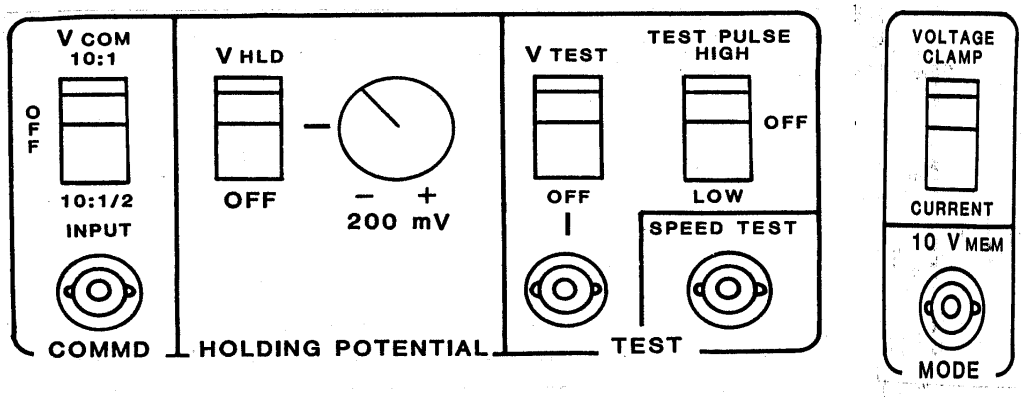


Figure 11: Test pulse and clamping controls

The Gain corrects or amplifies the scale factor of the output current. In the Probes section of the head stage, the dial is turned to 0.1G because the 8910 probe is used. Thus, in the Output section of the head stage, seen in Figure 12, the chosen gain must be divided by 100. For instance, if a Gain of 10 is chosen, then the output current (Iout) is amplified by 0.1 mV/pA.

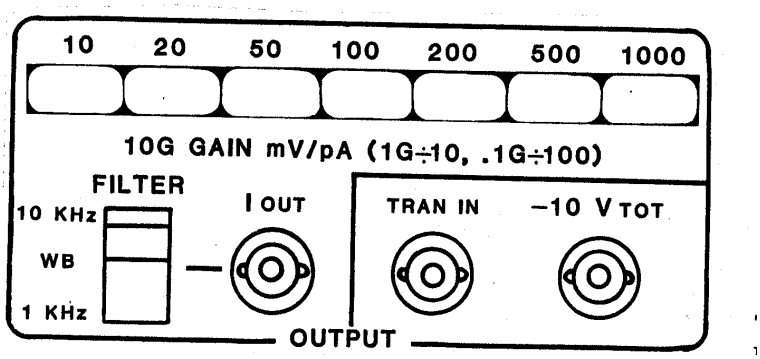

Figure 12: Output section of head stage

See the Dagan Operating Manual for more details on using this head stage [8].

In conjunction with the Dagan amplifier, the Tektronix 434 Storage Oscilloscope was used and connected to the head stage via the Iout location. With a voltage clamp, the voltage was read off of the oscilloscope monitor peak to peak and then converted to a current value using the 0.1 mV/pA conversion. Finally, Ohm's law was used to calculate the desired resistance as follows:

$$R = \frac{\text{test pulse voltage (corresponding to high or low)}}{\text{current square wave converted from voltage reading on oscilloscope}}$$

The resistance was measured using only the medium and measured again using cells. An increase in resistance should indicate that cells are successfully attaching to the electrodes.

## 3. EXPERIMENTS AND RESULTS

### 3.1 REACTIONS TO MATERIAL SAMPLES

Determining the proper materials to fabricate a cell-based biosensor was the main focus of this research. Due to the sensitivity of human embryonic kidney cells, I was able to determine how various materials affected the cells. Success of the material was determined by observing if the cells attached to the bottom of the dish or if they were floating. Floating cells indicated that the cells were dead and thus not compatible with the material.

During the process of splitting cells, I placed the cells into dishes containing medium and samples. Initially, I tested samples that contained a section of bare ceramic, Teflon covered ceramic, and ceramic covered in gold paste. After two days of allowing the cells to multiply and attach, I observed the cells. Using an inverted scope it was possible to see if the cells were living and attached to the bottom of the dish as well as the density of the cells in the dish. See experimental setup in Figure 13. Since I was not getting consistent results, I did this same experiment several times. Frequently the

density of attached cells was lower than expected implying that many to almost all of the cells in dish were dead. When there were living cells in the dish, I was unable to tell if the cells were attached only to the bottom of the dish or to the sample as well. On the dissecting scope, the roughness of the surfaces prevented me from being able to see the cells. Once I was certain that I was caring for the cells properly, I ran a series of tests to determine the exact cause of the death of cells. The following figure shows the experimental setup and the table summarizes the results:

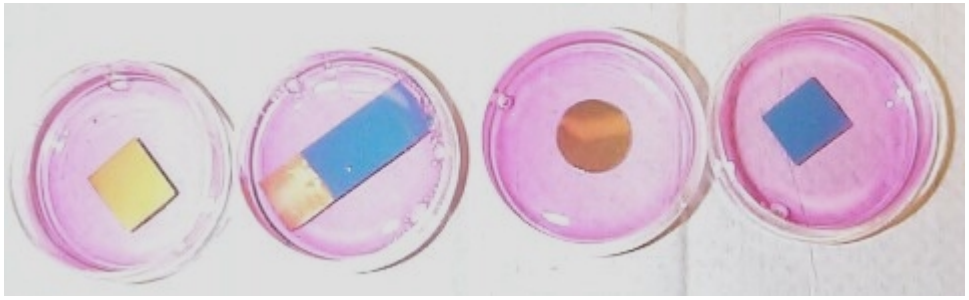

Figure 13: Experimental set up of material samples in medium with cells

| | Sterilization by Autoclaving | Sterilization by UV Light |
|---|---|---|
| Bare Ceramic | Living cells | Living cells |
| Teflon Covered Ceramic | Living cells | Living cells |
| Gold Paste on Ceramic | Dead cells | Dead cells |
| Gold Paste on Glass | Could not be fabricated | ------ |
| Pure gold on Glass | Living cells | ------ |
| Pure Gold on Ceramic | Living cells | ------ |

Figure 14: Table of Results

In the dishes that indicate living cell, the density of the cells on the bottom of the dish was approximately equal to the density on the bottom of the flask (the control). However, with the gold paste on ceramic, the density of cells attached to the dish was very low, much lower than in the flask. Additionally, many floating cells could be seen.

These experiments took several weeks because only a few dishes could be set up for each splitting of cells due to the given and needed concentration of cells. During these tests, each dish only contained a single type of sample. Additionally, after an individual splitting of cells, at least one dish contained living cells and the flask always contained a high concentration of healthy cells. Thus, there was never a “bad” batch of cells; the material caused the death of the cells.

Only glass was used in order to help visualize the cells since the dissecting scope was not useful. The glass slides, covered in a thin transparent layer of gold, allowed for use of the inverted scope. This revealed that with the use of pure gold on glass, a very high concentration of cells did indeed attach themselves to gold. A sample using gold paste on glass was made, but the glass could not withstand the cofiring process needed by the gold paste.

The table reveals that the gold paste is the culprit in respect to the death of the cells. Different ways of sterilization were attempted, but to no avail. I had initially hoped that the gold paste would be a suitable material for the electrodes due to its easy handling. However, after further research as well as contacting DuPont, I believe that the material properties of the thick film gold paste material, in conjunction with the Green Tape, are harmful to the cells. According to DuPont, the gold paste is primarily gold with inorganic materials. When the gold is cofired with the Green Tape, metal oxides and glass diffuse from the ceramic into the paste. A DuPont representative believes that the cells are most likely dying in response to the metal oxides. Although the metal oxides are also present in the green tape, green tape without the gold paste does not harm the cells. It is unclear why this is the case.

Pure gold was then deposited on the ceramics and tested. After its success with the cells, I tried to see the density of cells attached to the surface of the gold as opposed to the bottom of the dish via the SEM. Figure 15 shows cells attached to the gold on ceramics and to the gold on glass.

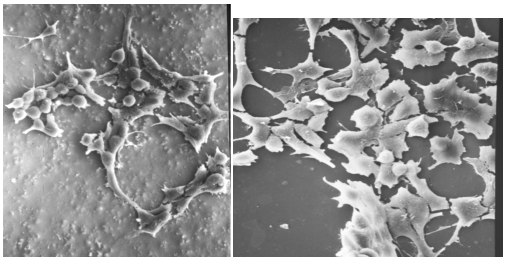

Figure 15: Cells attached to pure gold on ceramic (left) and glass (right), each at 640x magnification

The density of cells appears higher on the glass surface; however, the ceramic surface looked rough and the cells were difficult to focus on. It is possible that the density may have been higher than the SEM could reveal.

## 3.2 TESTING OF SENSOR

Figure 16: Setup of chamber, head stage and oscilloscope

The first fabricated biosensor was made with electrodes of gold paste covered in Teflon. The gold paste was exposed through a 125 μm hole. This sensor was used with the sea urchin eggs because they are not as sensitive to their surroundings and thus not killed by the gold paste. The resistance between each electrode in the chamber and an external electrode inserted into the bath was measured by filling the chamber with sterilized seawater. These measurements provided two different resistances depending on which electrode was used. One electrode revealed a resistance across the seawater of 50 kΩ while the other electrode showed a resistance of 400 MΩ.

Because this difference in values was obviously not what I was looking for, I tested the second device with the pure gold electrodes. I tested the resistance before removing a portion of the double-coated Teflon and exposing the electrodes to the seawater. With each electrode I found a resistance of 50 kΩ. Once I removed the Teflon by inserting a hole with a needle, I made resistance measurements again. No change in resistance occurred. By using a dissection microscope, I was able to see that the needle scratched the gold underneath the Teflon. This most likely proves that the needle successfully went all the way through the Teflon. From these results, I believe that the Teflon is significantly porous enough that removing a small portion does not affect the resistance. It is difficult to explain the difference of orders of magnitude in resistance measurements for the electrodes in the first biosensor.

Sea urchin eggs suspended in seawater were added to the chamber with the gold paste electrodes and resistance measurements were taken again. However, after trying several different methods of adding the eggs to the chamber, for instance putting just a pellet of jelly-free eggs onto an electrode, the measurements were identical to the resistance values recorded across seawater only. This may have occurred for several reasons. First, I had no way to verify that the eggs were actually attaching to the electrodes. If the hole had been covered, it may have only been sealed by debris due to the aforementioned problems in removing the jelly coat layer of the egg. Second, if the Teflon were in fact porous, an egg would not alter the resistance because a very large area of gold would be exposed to the medium and thus the current would go through the path of least resistance. Unfortunately, I have been unable to test the device with pure gold electrodes in the presence of cells due to lack of time.

Despite the inability to obtain a change in resistance when cells are present, the theoretical resistance has been determined. It has been noted that the typical resistivity of a neural axon is 1000 $\Omega cm^2$ [1] and for a Madin-Darby canine kidney (MDCK) cell the resistance is also 1000 $\Omega cm^2$ [9]. Thus, I have assumed these numbers as a first approximation of a general value for cell membrane resistance. By dividing the membrane resistance by the approximate attaching surface of the sea urchin egg (half the surface is of a sphere with radius 100 μm), the resulting resistance across an electrode sealed by an egg should equal 1.59 MΩ.

## 4. RECOMMENDATIONS

Further research and development of a cell-based biosensor can easily be continued using the results and knowledge gained. The next step in the research process is to perfect the device and the chamber by using materials that are more compatible. Pure gold should be used as the electrodes. It has been proven that the gold paste is damaging to the cells and should not be used. If cells are still having trouble attaching and living on the gold, alternatives can be used. For instance, a protein layer such as poly-l-lysine can be added to the electrode in order to induce the cells to attach. This step will depend on the type of cells being worked with. Many cells should have no trouble attaching without the protein layer.

In addition to increasing the quality of the materials used, further research should explore the efficiency of the Teflon as an insulating layer. Perhaps more than one layer can be coated on to the channel. If this does not appropriately insulate the surface of the chamber, other insulating materials should be explored.

Finally, using a needle to puncture a hole in the Teflon in order to expose a small surface area of gold was not sufficient. I could only manage to make the hole as small as 125 μm in diameter. Because most cells are smaller than this, including the relatively large sea urchin eggs, a laser should be used to expose an electrode of the appropriate size. The goal is to completely seal off the electron from the medium with the cell in order to gather measurements of the electrical activity across the cell's membrane via stimulating action potentials. However, if this cannot be successfully accomplished, impedance measurements can be made instead. In this situation, the electrode does not have to be completely covered. It just needs to be sufficiently covered in order to have the impedance of the cells dominating [2].

Sea urchin eggs will be the easiest to work with initially due to their size and robustness. Nevertheless, the methods used for removing the jelly layer must be perfected. Working with acidified seawater may prove successful with further attempts; however, many other methods can be used to remove the jelly layer, such as using collagenase. I recommend looking into alternative ways to care for the eggs.

After the physical design of the device and the caring of cells are perfected, successful electrical measurements can be made. Much research will have to go into how to trigger responses from the cells, but this research will be highly worthwhile. In the long run much information will be gained about how the various stimuli and environments affect a variety of cells.

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